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## Autophagy Related Ulk1 is Necessary for the Recovery of Mitochondrial Function after Skeletal Muscle Injury

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The objective of this study was to determine if autophagy is necessary for the recovery of mitochondrial function after muscle damage. Autophagy is a highly conserved cellular process for the degradation of dysfunctional or damaged organelles (e.g., mitochondria). We have previously demonstrated that traumatic muscle injury impairs mitochondrial content and is accompanied by an induction of autophagy. However, it is unclear: 1) if only traumatic muscle injury induces autophagy, 2) if autophagy induction is contingent on mitochondrial dysfunction, and 3) if autophagy is necessary for the functional recovery of muscle strength and mitochondrial function. To determine the relationship between muscle damage, mitochondrial function and autophagy induction, ten week old C57Bl/6 mice were randomly assigned to the following groups: eccentric contraction-induced injury (physiological), freeze injury (traumatic), or contractile fatigue (non-damaging control). Injured and contralateral uninjured tibialis anterior and extensor digitorum longus muscles were harvested at 0, 6 hours, 1, 3, and 7 days. Mitochondrial function was assessed via state 3 mitochondrial respiration rates from permeabilized muscle fiber bundles, and autophagy induction was measured via Beclin1, an autophagic protein activated downstream of Ulk1, and total LC3 protein content. There was no effect of contractile fatigue on mitochondrial function or autophagy induction at any time point ( $P > 0.19$ ). Eccentric contraction-induced injury did not elicit mitochondrial dysfunction in the injured muscle at any time point ( $P > 0.20$ ), but injured muscle did have greater Beclin1 content at 3 and 7 days (~2 fold,  $P = 0.03$ ). This suggests that physiological muscle damage may induce an autophagy response even in the absence of overt mitochondrial dysfunction. In contrast, freeze injury caused severe mitochondrial dysfunction immediately through 3 days after injury (16-53% of contralateral control,  $P = 0.04$ ), and was accompanied by a robust autophagy response in both Beclin1 and LC3 protein content (peak 7 at days ~40 fold ~14 fold  $P < 0.01$ , respectively) To determine if this robust autophagy response was necessary for the recovery of mitochondrial function, we subjected skeletal muscle-specific Ulk1 knockouts (Ulk1 KO) and their littermates controls (WT) to freeze injury and assessed the recovery of muscle strength and mitochondrial function at 7 days. There was no difference in muscle strength between Ulk1 KO and WT mice prior to injury ( $P = 0.47$ ), but at 7 days muscle strength in Ulk1 KO mice had recovered to only 12% of preinjury strength, significantly less than WT mice (30%,  $P < 0.01$ ). There was no difference in mitochondrial function in the uninjured muscle from Ulk1 KO and WT mice ( $P = 0.35$ ), although at 7 days, mitochondrial function was an astonishing 32% of uninjured in ULK1 KO mice compared to 64% of uninjured in WT mice ( $P = 0.01$ ). In conclusion, autophagy is induced following both traumatic and physiological muscle injury and Ulk1 is required for the recovery of mitochondrial function after traumatic muscle injury.